# STUDIES ON THE MORPHOLOGY OF *ESCHERICHIA COLI* CELLS EXPOSED TO PENICILLIN

BY

ARTHUR JAMES SHANAHAN

B. S., Holy Cross College, 1941

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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R. UNIV. BIBLIOTHEEK LEIDEN

# STUDIES ON THE MORPHOLOGY OF *ESCHERICHIA COLI* CELLS EXPOSED TO PENICILLIN

There is an abundant literature on the subject of bacterial pleomorphism. The literature may be divided roughly into two categories. First, it has been repeatedly shown that morphological changes may occur when bacteria are grown on media containing an excess of chemicals such as salts, dyes and penicillin. Altering the pH of a medium is also known to produce changes in bacterial morphology. Secondly, naturally occurring pleomorphic forms have been observed in cultures of numerous genera of bacteria and especially in cultures of the pleuropneumonia-like organisms.

In the present studies, fusiform bodies or large, swollen, bulbous cells were of principal interest. Such structures have been observed in cultures of bacteria and pleuropneumonia-like organisms but their nature and viability have not been fully determined. When fusiform bodies were found in a culture other abnormal forms were also usually present. A second pleomorphic form frequently encountered in bacterial cultures is a large, round cell referred to as "balloon" form, swollen large body or Kuhn's A-form. These types of pleomorphic cells have been observed mostly when inducing agents have been added to media but Dienes and others have found similar structures in pleuropneumonia-like organisms under normal growth conditions. The large round bodies apparently have a dual nature, according to Dienes, since some produce normal bacteria by segmentation and formation of granules while others develop into Klieneberger's L1 type colonies.

## Experimental

Nutrient broth cultures of *Escherichia coli* to which

various concentrations of penicillin* were added were observed for resistance of each strain and for changes in morphology. Variation in resistance to penicillin of most strains of *Echerichia coli* tested coupled with difficulty in obtaining satisfactorily stained preparations prompted use of agar in place of broth media. Although pleomorphic cells appeared in broth cultures with penicillin they were fewer in number and occurred less regularly than in penicillin agar cultures.

MacConkey's agar (Difco) was selected for use in most of the tests reported. Appropriate amounts of penicillin were added to melted agar after diluted broth cultures of *Escherichia coli* had been inoculated, then the agar was poured into sterile culture dishes. After incubation at 37°C. for various lengths of time typical colonies were selected and Gram stains made. Examination of 23 strains of *Escherichia coli* revealed that all strains responded to penicillin with production of abnormal cells including: partially divided rods; fusiform structures; spindle-shaped cells; long, thin filamentous rods; swollen, elongated rods; yeast-like cells and occasionally coccal forms. With subinhibitory concentrations of penicillin normal short rods were frequently eliminated but usually were present in cultures with lower concentrations of penicillin.

When serial transfers were made on penicillin agar the organisms were found to increase in resistance to penicillin with a corresponding disappearance of pleomorphic forms. Then if the concentration of penicillin was increased, abnormal cells re-appeared in the next transfer indicating the necessity of subinhibitory concentrations for production of pleomorphism. The above procedures were applied to several strains of *Escherichia coli* with like results in each case. All cells remained gram-negative and further development of abnormal forms was not observed. Nutrient agar

*Na-penicillin used in these studies was supplied by the Schenley Laboratories, Lawrenceburg, Indiana.

and lactose agar did not yield as many pleomorphic forms as were found on MacConkey's agar when several strains were exposed to various concentrations of penicillin.

A modified warm-stage method for culturing *Escherichia coli* was adopted with considerable success. Examination of organisms growing on MacConkey's agar containing penicillin was made using an oil immersion lens. Frequent observations made it possible to determine that a fusiform body originated near a dark area at about the middle of a cell and became a bulbous structure by simple, direct swelling. In most cases a fusiform structure was preceded by formation of a long, thin filamentous rod in which there was no indication of fission. Twenty-three strains of *Escherichia coli* were examined by warm-stage methods and all strains were remarkably alike. Young bulbous cells were homogeneous in cellular content but older cells became quite granulated. Further development of fusiform bodies was not observed when they were transferred to media not containing penicillin.

Warm-stage cultures of several strains of *Escherichia coli* grown on lactose agar revealed the presence of large round bodies similar to those reported by others when various substances were added to media. Although fusiform bodies were also present in lactose agar cultures they were few in number and difficult to find. Large, swollen round cells noted in two strains resembled "balloon" forms reported by Dienes and others. No further development of these cells could be observed but they often disintegrated or autolyzed.

Alkaline reversion was frequently noted when various strains of *Escherichia coli* were cultured on MacConkey's agar containing penicillin. Fusiform bodies and other abnormal cells did not appear in alkaline media and a slight acid pH was found favorable for production of pleomorphic cells. Penicillin-treated *Escherichia coli* cells apparently survived long incubation (18 days) at 37°C. better than control cultures with no penicillin in the medium. No

explanation can be offered for these results. Cultures of three strains of *Escherichia coli* were found to produce abnormal cells, resembling those observed in penicillin-treated cultures, when they were held at 10-14°C. on MacConkey's agar not containing penicillin. Further investigations of this phenomenon were not made.

Electron micrographs and light microscope photographs of penicillin-treated *Escherichia coli* cells were presented. Granulation of fusiform bodies and other swollen cells was well brought out in the electron micrographs. Photographs made of living and stained preparations did not show granulation as well as electron micrographs.

## Discussion and Summary

When subinhibitory concentrations of penicillin were added to culture media, strains of *Escherichia coli* were found to produce several pleomorphic types of cells. Abnormal forms produced included: partially divided rods; swollen filamentous rods; fusiform bodies; spindle-shaped cells and large, round bodies.

A study of fusiform structures and large round bodies was made (1) by observation of living cultures growing on MacConkey's agar and lactose agar; (2) by examination of stained preparations and (3) with the electron microscope. Granulation of fusiform and swollen cells produced under the influence of penicillin appeared to be a characteristic of penicillin action. No further development of fusiform and large round bodies could be observed by any methods employed.

## VITA

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